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(54) Title: A METHOD OF FORMING A DYNAMIC COMBINATORIAL LIBRARY USING A SCAFFOLD

(57) Abstract: The present invention relates to a method of forming a library of components which are potentially capable of binding to a target, which method comprises i) selecting a plurality of molecules carrying a functionality which may interact with a binding site of the said target, said molecules furthermore having a linking group which is capable of interacting with other linking groups under the formation of reversible bonds; ii) selecting a scaffold carrying two or more linking groups which are capable of interacting with the linking groups on the compound carrying a functionality; iii) reacting the scaffold and the molecules carrying the functionality in the presence of the target, under conditions where a formation of reversible bonds between the functionalities on the scaffold and on the molecules carrying a functionality occurs. In one embodiment of the present invention, the scaffold is designed to potentially interact with a binding site of known structure in a known target. The design, or pre-tailoring, of the scaffold will occur with respect to its size, three dimensional structure and/or flexibility. In a further embodiment of the present invention, the scaffold is designed to permit a screening against targets having a binding site of unknown structure, in particular to find components which are active in protein-protein-interactions.

A method of forming a dynamic combinatorial library using a scaffold

The present invention relates to dynamic combinatorial chemistry (DCC), more particularly to the use of scaffolds in the screening method for finding active components (lead compounds) by DCC. In one embodiment of the present invention, the scaffolds are pre-tailored for a target of known structure for which active components are sought. In a further embodiment of the present invention, the scaffolds are not pre-tailored, but are of a structure permitting their use in DCC-screening processes against targets of unknown structure.

New chemical or biological entities with useful properties are classically generated by identifying a chemical or biological compound (a so-called lead compound) with some desirable properties or activities, creating varieties of said compound to form a library, and evaluating the properties and activities of those variant compounds.

The conventional approach is limited by the relatively small pool of previously identified compounds which may be screened to identify new compounds with the desirable property or activity.

Another drawback pertains to the step of the creation of variants. Traditionally, compound variants are generated by chemists or biologists using a conventional chemical or biological synthesis procedure. Thus, the generation of compound variants is time-consuming and requires huge amounts of work.

To assist in the generation of new chemical compounds, attention has recently turned to the use of combinatorial chemical libraries.

Combinatorial chemistry (CC) has experienced an explosive growth in recent years. It provides a powerful methology for exploring the molecular geometrical and interactional spaces through molecular diversity generation. This is in particular the case for the discovery of new biologically active substances and medical drugs. It resides on the constitution of vast combinatorial libraries (CLs), extensive collections of molecules derived from a set of units connected by successive and repetetive application of specific chemical reactions. It is thus based on a large population of different molecules that are present as discrete entities.

The constitution of a CL of components amounts to the fabrication of a large collection of components. The CL is then screened against a target, with the goal that one of its constituents will fit the target lock/receptor, i.e. show an activity, and be retrievable from the mixture.

In contrast to this, the present invention makes use of the so-called dynamic combinatorial chemistry which is a conceptionally different approach. It relies on a reversible connection process for the spontaneous and continuous generation of all possible combinations of a set of basic constituents, thus making virtually available all structural and interactional features that these combinations may present. Such multicomponent self-assembly amounts to the presentation of a dynamic combinatorial library (DCL) which is a potential library made up of all possible combinations in number and nature of the available components. By recruiting the correct partners from the set of those available, the component, among all those possible, that possesses the features most suitable for formation of the optimal supramolecular entity with the target site is selected. The composition of the set of components/subunits depends on the extent to which the possible combinations cover the geometrical and interactional spaces of the targets.

Self-assembly in a multi-component system is a combinatorial process with a search procedure directed by the kinetic and thermodynamic parameters imposed by the nature of constituents and their interactions.

In WO 97/43232 there is disclosed a substance library which library consists of molecular species which are bonded to a molecular pairing system. The pairing system is composed of molecules, in the preferred embodiment, which are selected amongst specially designed nucleic acids which can bind to each other in a certain manner which results in a particular geometric form. The molecular species are selected, in a preferred embodiment, from the group consisting of peptides, and these peptides are designed according to the particular requirements of a given component which is brought into contact with the library component. The complex which forms upon contact with the component is identified, in order to evaluate the interaction between a component and complex.

In Proc. Natl. Acad. Sci. USA 1997 (94), 2106, Ivan Huc and Jean-Marie Lehn disclose a method for the generation of a dynamic combinatorial library of imines from structural fragments bearing aldehyde and amino groups. The method is directed toward the synthesis of inhibitors of the enzyme carbonic anhydrase by recognition-involved assembly, and the synthesis of the above-mentioned imines is carried out in the presence of the said enzyme carbonic anhydrase. It was found that reversible combination of the used amines and aldehydes leads to the shift of the equilibrium population towards the imine product that was closest in structure to a known highly efficient inhibitor of the enzyme.

The application WO 01/64605 discloses a process for establishing a dynamic combinatorial library for a target which binds at least two functionalities. The method comprises selecting a plurality of molecules carrying functionalities which, by combination with each other, are capable of forming an entity which may bind to the functionalities in the target. The molecules carrying the functionalities are linked by a spacer group which allows a reversible formation and cleavage of bonds. The cleavage and formation of the bonds can occur in the spacer group, or at the terminus of it. Generally, the spacer group will be selected such as to potentially fit into the binding site(s) of the target. The spacer groups disclosed in the application are all linear entities like for example alkane derivatives, and contain a maximum of two linking groups allowing the formation and cleavage reversible bonds.

In all patent application in the field of DCC which have been filed up to now, as well as literature published in this field, DCC-screening methods are disclosed which for the generation of the library employ molecules having a "functionality" which can bind to the target. The molecule carrying the functionality furthermore has a "linking group" forming reversible bonds with the linking groups present on another compound, in the course of the screening process. Due to the reversibility, the bond formed by the reaction between two linking groups can be cleaved, thereafter a new component can be formed by a new reaction with the linking group present on a further molecule having a functionality.

The concept of DCC is proved only for the formation and cleavage ("scrambling") round one or two reversible bonds which are each formed by the reaction or a supramolecular interaction between the two linking groups. The dynamic libraries which are generated are, in consequence, of limited size with respect to the number of constituents. Furthermore, in the constituents of the library a maximum of two functionalities can be varied.

As is clear from the foregoing, it has, up to now, only been possible to establish combinatorial libraries with compounds which have a maximum of two functionalities which can bind to the target or interact with it otherwise. In some cases, this may be a limitation of the DCC screening method, e.g. in cases where more complex molecules are sought for. It would be desireable to have a method which allows the generation of virtual libraries of a compound which contains more at least two, preferably more than two, functionalities which can interact with the target, for example bind to it.

A further limitation of the DCC-processes known until now is that the building blocks which bind to each other in the screening process are selected according to their potential ability to bind to a given target of known structure or, respectively, having a binding site of known structure. There exist however targets of medical interest the structure of which is not known or not sufficiently elucidated in order to allow a selection of the start components for a DCC-screening process.

It is the object of the present invention to create a method which overcomes the limitations of the prior art process. It would in particular be desirable, as a first object, to have a DCC-

method which allows the variation of at least two, preferably more than two, functionalities which may bind to the target and hence allows the generation of vast virtual libraries. It is a further object of the present invention to create a method which allows to carry out DCC-processes, i.e. finding an active component for a given target, by a self-assembly-process which is appropriate to be used against a target of unknown structure or having a binding site of an unknown structure, in particular for the finding of components active in protein-protein interactions.

These objects are attained by a method of forming a library of components which are potentially capable of binding to a target, which method comprises

i) selecting a plurality of molecules carrying a functionality which may interact with a binding site of the said target, said molecules furthermore having a linking group which is capable of interacting with other linking groups under the formation of reversible bonds;

ii) selecting a scaffold carrying two or more linking groups which are capable of interacting with the linking groups on the compound carrying a functionality;

iii) reacting the scaffold and the molecules carrying the functionality in the presence of the target, under conditions where a formation of reversible bonds between the functionalities on the scaffold and on the molecules carrying a functionality occurs.

In one embodiment of the present invention, the scaffold is designed to potentially interact with a binding site of known structure in a known target. The design, or pre-tailoring, of the scaffold will occur with respect to its size, three dimensional structure and/or flexibility.

In a further embodiment of the present invention, the scaffold is designed to permit a screening against targets having a binding site of unknown structure, in particular to find components which are active in protein-protein interactions.

The present invention is also drawn towards a method of assessing the binding capacity of a component to bind to a given target, which method comprises the steps i) to iii) carried out as described above, and additionally

iv) identifying the components which preferably bind to the target.

Still, the present application is drawn towards the libraries created with the method according to the present invention.

Thus, in the first embodiment, the DCC-screening method is carried out against a target of known structure, respectively a target having a binding site of known structure, using a scaffold which carries at least two, preferably two to six, more preferably two to four, in particular three or four linking groups which can react with linking groups which are present on the molecules carrying the functionalities.

The term "functionality" used in the present invention designates a unit which can bind to the binding sites of the target, or interact with the target otherwise than by binding to it, for example by steric interactions.

"Functionality" means any polar, nonpolar, hydrophilic or lipophilic, or charged unit or subunit or electron donor or electron acceptor group. "Functionality" on the one hand includes simple functionalities like amino and imino groups and derivatives thereof, hydroxy and mercapto groups and derivatives thereof, oxo and thioxo groups, formyl and thioformyl groups, aryl groups, substituted aryl groups, phenyl groups, substituted phenyl groups, pyridyl groups and derivatives thereof, carboxy groups and carboxylato groups and derivatives therof, alkyloxycarbonyl groups, (di)thiocarboxy groups and derivatives thereof, (di)thiocarboxylato groups, carbamoyl groups and derivatives thereof, sulfo, sulfino and sulfeno groups and derivatives thereof, alkyloxysulfonyl, alkyloxysulfinyl and alkyloxysulfenyl groups, sulfamoyl, sulfinamoyl and sulfenamoyl groups and derivatives thereof, cyano and (iso)(thio)cyanato groups, hydroperoxy groups, nitroso groups, hydroxyamino groups, hydrazino groups, $-NR^1R^2$, $-^+NHR^1R^2$ and $-^+NR^1R^2R^3$ groups, wherein R^1, R^2, and R^3 are identical or different and represent alkyl, cycloalkyl,

alkylcycloalkyl, aryl, alkylaryl with 1 to 40 C atoms, $-^{+}OR^{1}R^{2}$ groups wherein R^1 and R^2 are identical or different and represent alkyl, cycloalkyl, alkylcycloalkyl, aryl, alkylaryl with 1 to 40 C atoms, hydrazide groups and any other suitable groups known to a person skilled in the art.

On the other hand, "functionality" also includes more complex components, and non-limiting examples include heterocycles carrying one or more heteroatoms in the ring selected from the group consisting of N, O and S, amino acids and oligo- and polypeptides, sugars (preferably hexoses and pentoses), sugar derivatives (like peracetylated sugars) and oligomers and polymers thereof, and nucleic acids and derivatives thereof.

The functionalities can thus be linked to a molecule, i.e. a molecule carrying the functionality (functionalities) is formed; the functionality can also itself be the molecule (and thus form the molecule carrying the functionality). An example of the latter case is a sugar molecule.

A molecule carrying a functionality furthermore carries at least one "linking group" which term designates a group capable of reacting with another linking group, which may be identical with or different from the first linking groups, under the formation of a reversible chemical bond. Such reversible bond can be covalent or supramolecular. The molecules carrying a functionality are the building blocks from which the (active) components are formed.

Examples for linking groups which may react with other linking groups under reversible bond covalent formation include amino groups, aldehyde groups, keto groups, thiol groups, olefinic groups, alcohol groups, carbonyl groups, hydrazine groups, hydroxylamine groups and borate groups. Examples of reversible covalent reactions with the above-mentioned groups involved are those where carbonyl groups react under the formation of imines, acylhydrazones, amides, acetals, and esters. In particular, the reaction of amino groups with carbonyl groups to imines, oximes or hydrazones is useful. Reactions such as thiol exchange in disulphides or alcohol exchange in borate esters are further examples for preferred embodiments, as well as reversible Diels-Alder and other thermal- or

photoinduced rearrangements like sigmatropic and electrocyclic rearrangements, and Michael reactions or alkene metathesis using catalysts that may be soluble in water. Photoinduced interconversions represent another possibility leading to photodynamic combinatorial processes.

Dynamic libraries can also be of conformational or configurational character, for example in cis-trans isomerisation, where the difference in configuration can be used in the selection. Conformational dynamic processes are e.g. internal rotation or ring and site inversion. Other suitable library components, reversible processes and biological targets will be known to a person skilled in the art.

When the molecules carrying a functionality and the scaffold combine with each other, "components" are formed. These components may already present the "ligands" which have two or more functionalities and which bind to the target. They may also only present precursors of the ligands. That means that another reaction must take place to transform the components to the ligands like, for example, the reduction of generated imines to amines. The ligands as well as the ligand precursors will in the present invention generally be referred to as "components". The difference between a ligand and its precursor can however often not be determined unambigously. This becomes evident for example in the case of imines/amines: in the screening process, imines are generated the generation of some of which is favored by the interaction with the target, meaning that at least a certain degree of binding has taken place. When the imines are converted to the relating amines, by reduction, then the amines derived from the most active imines (i.e. those formed in the highest amounts) also generally show the highest activity. It is thus not clear, in this case, which of the two species is the ligand and which the precursor.

Component (ligand) means a molecule with a molecular weight typically not greater than 1500, preferably not greater than 1000, advantageously not greater than 500, which possesses an affinity for a target, i.e., that is able to interact with the target by forming one or a plurality of weak bonds such as hydrogen bonds, hydrophobic interactions, charge-charge interactions, Van der Waals interactions, donor-acceptor interactions, charge-transfer interactions, metal ion bindings, etc. The ligands generated with the method

according to the present invention generally have at least two functionalities, preferably at least three functionalities, which are able to interact with the "target".

"Target" means a biological or synthetical macromolecule with a molecular weight typically greater than 5000. Biological macromolecules may be proteins including lipoproteins, glycoproteins and analogues of proteins, wherein either the peptide bond –CO–NH– is replaced by an analogous bond, possibly reversible such as an imine, ester, sulfonamide, sulfone, sulfoxide, phosphate, phosphonate, phosphonamide, guanidine, urea, thiourea, or imide bond, or wherein the aminoacids are replaced by synthetical derivatives thereof. The natural proteins may have differing functions, they may act namely as enzymes, as receptors or as antibodies. Receptors may be membrane receptors, hormone receptors, or signal transducers.

If the target is an enzyme, the ligand which is sought to be obtained may act as a substrate, an inhibitor or an activator for said enzyme.

If the target is a receptor, the ligand which is sought to be obtained may act as a natural or artificial ligand, an agonist or an antagonist for said receptor.

If the target is an antibody, the ligand which is sought to be obtained may act as an antigen for said antibody.

The scope of the present invention is not limited to biological targets. Any natural or synthetic organic and inorganic target may be used. In general, any kind of target for which an activity assay exists, is suitable. The activity may e.g. be determined, by measuring the change of fluorescence, viscosity, conductivity or IR or UV absorption. Therefore suitable targets, besides those cited above may be zeolithes, clathrates, oligonucleotides, oligopeptide, oligosaccharides, sensors, clusters, RNA aptamers, organic and inorganic catalysts, ionophores, any kind of macrocycles like metallomacrocycles, macrocyclic lactams, macrocyclic esters, macrobicyclic cryptands and macrocyclic oligocholates, any kind of synthetic polymers like polyaminoacids, polyamides, polyesters, polyalcohols and

mixtures thereof, etc. Ligands may have any kind of linking groups mentioned above. Even simple "molecules" like cations or anions may act as ligands.

In general, all kinds of molecules, the one of which can act as a ligand and the other one as a target, are suitable to be used in the method according to the present invention.

"Reversible" refers to bond formation and cleavage in a dynamic equilibrium. Reversible bonds not only include reversible covalent reactions, but also reversible connection processes involving non-covalent, supramolecular interactions, such as metal ion coordination, π-stacking, hydrogen bonding, or charge-charge interactions.

The molecules carrying a functionalty and linking groups (the building blocks) are then brought into contact with the scaffold, in solution, and under conditions where a reversible formation of bonds by a reaction between the linking groups present on the scaffold and on the molecules occurs. Such a reaction can be the formation of a covalent chemical bond or a supramolecular bond. The reaction is generally continued until equilibrium is reached. The reaction can be carried out in the absence or in the presence of the target. By this process, a dynamic combinatorial library (DCL) is generated. Such library contains all possible combinations of the molecules carrying a functionality with the scaffold, and hence is a potential library made up of all possible combinations in number and nature of the available components. This is followed by the identification of the component(s), among all those possible, that posesse(s) the features most suitable for formation of the optimal supramolecular entity with the target site, by recruiting the correct partners from the set of those available. The degree of completeness of the set of components/subunits depends on the extent to which the possible combinations cover the geometrical and interactional species of the targets.

The scaffold can be any molecule which carries at least two linking groups, preferably at least three linking groups, and which has a structure which can potentially form the basis of an active component for a given target. In order to transform the scaffold into a component, the functionalities have to be attached to the scaffold. In a DCC-screening process, this occurs via the formation of reversible bonds by the interaction of linking

groups on the molecule carrying the functionality and the scaffold. The scaffold can itself already have a certain activity with respect to the target, in general a biological activity. The scaffold can also show no activity. However, in any case, it is only after the attachment of functionalities to the scaffold that a lead compound, having a desired activity, is generated.

In one embodiment of the present invention, the scaffold is selected according to the requirements given by the binding site of the target. These requirements can be with respect to a certain size, three-dimensional structure and/or flexibility of the scaffold. It is mandatory that the scaffold can be easily controlled and operated under mild conditions. Furthermore, the compatibility with the bonding of the functionality to the target is an important feature of an appropriate scaffold for a given target. By the right choice of a scaffold having an appropriate size, three-dimensional structure and/or flexibility, the compounds formed, respectively the functionalities on the compounds, will fit into the binding site of the target and interact with it. One method to establish the appropriate size of the scaffold is, for example, analyzing the size of the binding site by methods known in the art like, for example, by X-ray structural analysis. It is also possible, for example, to evaluate the size of a known ligand for a given binding target and mimic this size.

The scaffold is a small organic or inorganic molecule. Organic molecules are preferred. In the context of the present invention, the term "small molecule" denotes a molecule which has a molecular weight in a region typical for organic molecules having a pharmacological activity and which are synthesized by chemical methods or are accessible by chemical methods. Preferably, the small molecules have a molecular weight of below about 500. The term "small molecule" does not refer to molecules of a high molecular with a pharmacological activity and which are typically synthesized by biochemical methods.

"Organic molecules" are molecules which contain at least one unit in the structure which is derived from the units $-CH_3$, $-CH_2-$ or $\equiv CH$ wherein the hydrogen can partially or totally be replaced by substituents which can form a bond to the remaining carbon atom. Such substituents are known to the person skilled in the art and include, for example, halogen

atoms, amino groups, ogygen atoms, hydroxy, alkoxy and aryloxy groups, thiol groups, organophosphine groups and organosilicon groups.

The organic molecule representing the scaffold can be of a cyclic or non-cyclic structure and can contain one or more heteroatoms, preferably from the group consisting of N, O, S, P, B, Si and halogens. The scaffold can also contain one or more double or triple bonds. The scaffold contains in its hydrocarbon chain at least one carbon atom, preferably at least three carbon atoms, in particular four to ten carbon atoms. The scaffold furthermore carries at least two linking groups, preferably two to six linking groups, more preferably two to four linking groups, in particular three or four linking groups which are selected from the group cited above in connection with the molecules carrying the functionalities.

The linking groups which are present on the scaffold are preferably selected from the group consisting of thiol groups, aldehyde groups, keto groups, olefin groups, amino groups, hydrazine groups and borate groups.

The scaffold can react with the molecules carrying a functionality. There will thus be attached linking groups to the scaffold, which linking groups can react with linking groups present on the molecules carrying a functionality. The linking groups do not belong to the scaffold, as the term is understood in the context of the present invention. The scaffold can have one or more functionalities which are in accordance with the definition given above. The functionalities will not react with the linking groups on the molecule carrying a functionality. The scaffold can thus also be regarded as a molecule carrying a functionality. The scaffold is also a common building block of the components formed in the course of the generation of the library. The library consists of the building blocks and the components formed.

In a preferred embodiment, the scaffold is an oligopeptide or an oligopeptide derivative having a peptide-like three-dimensional structure. In a further preferred embodiment, the scaffold is an aromatic or non-aromatic cyclic hydrocarbon molecule, or it is based on an aromatic or non-aromatic cyclic hydrocarbon molecule. The cyclic molecule is preferably selected from the group consisting of mononuclear cyclic compounds and fused bi- to

quadronuclear cyclic compounds. All cyclic compounds mentioned above can contain one or more heteroatoms from the group consisting of N, O, and S.

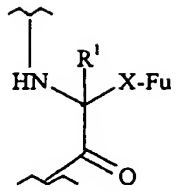
In one more preferred embodiment, the scaffold is a 4 to 7-membered aromatic or non-aromatic ring which can contain in its cycle up to three hetero atoms from the group consisting of O, S and N, respectively a molecule based on such structure. It is particularly preferred if the scaffold is an aromatic or non-aromatic 6-membered carbocyclic ring.

A further object of the present invention is a DCC-method which allows to screen against targets of unknown structure. An important embodiment thereof is the identification of compounds which show an activity in protein-protein interactions, with inhibitors of protein-protein interactions being of particular importance. Currently, there exists no efficient solution to this problem in classical drug discovery.

The main difficulty with finding drug candidates that can bind to protein surface is the competition with the large protein-protein interaction areas which are often in the range of hundreds of square angstroms. Furthermore, some known protein and peptide-surface binders are fairly large molecules. Due to this, the bioavailability of these large molecules is impaired.

In a further embodiment of the present invention, there are thus provided scaffolds which can be used in the generation of dynamic combinatorial libraries. The scaffolds carry at least two linking groups capable of forming a reversible bond by the the reaction with other linking groups. The scaffolds lend themselves for the screening against targets of unknown structure and to find lead structures, in particular for the finding of components active in protein-protein interaction.

These compounds are cyclic oligopeptides. In a preferred embodiment, the cyclic oligopeptides contain in their cycle 3 to 7 peptide units of which at least two units are, independently of each other, in accordance with formula (I)



I

in which

Fu is a linking group selected from -C(O)H, -C(O)R^2, -C(O)OM, -CR^3=CH_2, -C≡CH NHR^4, - N(R^6)-NHR^5, - SH, -OH and -$B(OH)_2$, and which linking groups Fu are identical or different from each other in case two or more of them are present in the cyclic oligopeptide;

X is a single or a double bond or a C_1-C_5-alkylene group in which one or more methylene groups can be replaced by O, S or NR^7;

R^1 is H or a C_1-C_4-alkyl group; or R^1 forms together with the N-atom in beta-position a 5- to 6- membered heterocycle under replacement of the H-atom attached to the said N-atom:

R^2 is a C_1-C_6-alkyl group;

R^3 is selected from the group consisting of H; C_1-C_{10}-alkyl; phenyl, -CH_2(phenyl), -CH_2CH_2(phenyl), phenoxy, heteroaryl -CH_2(heteroaryl), -CH_2CH_2(heteroaryl) and heteroaroxy;

R^4, R^5 and R^6 are independently of each other selected from the group consisting of H; unsubstituted and at least monosubstituted C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl and C_2-C_{10}-alkynyl, the substituents of which are selected from the group consisting of halides, OH, C_1-C_6-alkoxy, (C_1-C_6-alkyl)mercapto, CN, $COOR^8$, $CONR^9R^{10}$, unsubstituted and at least monosubstituted phenyl and heteroaryl, the substituents of which are selected from the group consisting of halides, pseudohalides, C_1-C_3-alkyl, C_1-C_3-alkoxy and CF_3; and unsubstituted and at least monosubstituted phenyl and heteroaryl, the substituents of which are selected from the group consisting of halides, pseudohalides, C_1-C_3-alkyl, C_1-C_3-alkoxy and CF_3;

R^7 is H or a C_1-C_6-alkyl group;

R^8 is H, C_1-C_6-alkyl or benzyl;

R^9 is selected from the group consisting of : H; C_1-C_6-alkyl which can be phenyl-substituted; and phenyl; and wherein each of the aforementioned aromatic groups can be unsubstituted or carry one or more substituents from the group consisting of halides, pseudohalides, C_1-C_3-alkyl, C_1-C_3-alkoxy and CF_3;

R^{10} independently has the same meaning as R^9.

M is selected from the group consisting of H; alkali metals; alkaline earth metals and ammonium groups which may carry one or more organic substituents.

In case X is a single or double bond, the group Fu is directly linked with the carbon atom in the cycle.

Preferably, at least three identical or different linking groups Fu are present in the cyclic oligopeptides according to the invention. In particular, three to four groups Fu which can be identical or different are present.

In a preferred embodiment of the present invention, the cyclic oligopeptides according to the invention contain 4 to 6 peptide units in the cycle, in particular 5 peptide units.

The peptide units can be identical or different from each other. When the units are different, they can all be different from each other, or two or more can be different from each other.

The peptide units are derived from amino acids which are known to the person skilled in the art. They are in particular derived from the amino acids occuring in nature. Examples for suitable amino acids include glycine, alanine, valine, leucine, isoleucine, asparigine, aspartic acid, glutamic acid, proline, lysine, serine, threonine and cystein.

The peptide units can also be derived from artificial amino acids not occuring in nature.

The term "peptide unit" used in the content of the present invention designates a unit which is derived from an amino acid, in which one amino group forms an amide function with the

carboxyl function of another peptide unit, and one carboxyl group forms an amide function with the amino group of another peptide unit. The peptide unit can contain linking groups Fu, which groups can be already present in the amino acid from which the peptide unit is derived or which are introduced by clinical or biological synthesis.

The peptide units in the cyclic oligopeptides according to the invention can be in the D- or L- form.

In case the cyclic oligopeptides according to the present invention contain linking groups Fu which do not occur in the amino acids as such, these groups are introduced into the starting amino acids by methods known to the person skilled in the art. The linking groups are the same as those which can be used on scaffolds designed for targets of known structure. These linking groups have been cited beforehand.

Cyclic oligopeptides represent a viable compromise between the molecular weight and the coverage area. The macrocycles can, for example, be designed to have a slightly concave shape with all side chains pointing toward the target protein surface. Further properties of the cyclic oligopeptides can be modulated by the choice of the respective amino acids. For example, the presence of the one to two proline residues increases rigidity of the macrocycles, while the presence of the aspartic acid unit improves solubility and renders the structure detectable by electrospray MS. The proteolytic stability of the cyclic oligopeptides and also of the active compounds obtained after the screening process can often be modified by the choice of the stereochemistry of the compounds.

The cyclic oligopeptides according to formula I are preferably used in finding molecules active in impairing protein-protein interactions, in particular for finding inhibitors of protein-protein interactions, by DCC. In this process, ligands are identified that bind to the surface of a single protein subunit in such a mode that they compete with the binding of their other protein counterpart.

The process comprises employing the cyclic oligopeptide as a scaffold for the reversible attachment, by covalent or supramolecular bonds, of compounds belonging to a preselected dynamic pool of compounds which are supposed to interact with the protein in question. These compounds are molecules which carry a functionality and, furthermore, at least one linking group which reacts with the linking groups Fu present on the cyclic oligopeptide in a reversible manner. The molecules carrying the functionality are, of course, of the same

type as those mentioned above, i.e. having a functionality of the type described above which can interact with the binding site in the target. The molecules furthermore contain at least one linking group of the type mentioned above to form a reversible bond with the linking groups on the scaffold. In this process, an interaction between the cyclic oligopeptide scaffold, the dynamic pool of compounds and a target, preferably a protein, takes place.

The DCL's of potential protein surface ligands are formed by interaction of the cyclic oligopeptide used as a scaffold and screened for affinity by any technique previously described for DCC and known to the person skilled in the art.

Preferably, the scaffolds used in the present invention allow to lock the process by a simple reaction or by changing conditions of the equilibrium. This applies both to scaffolds for screening against targets of known and of unknown structure. When designing a dynamic library making use of the method according to the present invention, two different procedures can be employed. In one procedure, library generating and screening is performed in one single step; in the other procedure, these two steps are carried out separately. The particulars for the above-mentioned different procedures are the following:

a) adaptive, combinatorial libraries (one-step procedure): the generation of the library is conducted in a single step in the presence of the target, so that the library composition may adjust leading to selection and amplification of the preferred component(s); the DCL may be real or virtual; screening by the target occurs in parallel with the reversible generation of the library constituents; this is the approach where the dynamic features are operative over the whole process;

b) pre-equilibrated dynamic combinatorial libraries (pDCL) (two-step procedure): the libraries are generated by reversible interconversion and equilibration in absence of the target, which is added in a second step. The addition of the target can occur to the library which is still fully active, i.e. which is still kept under equilibrium conditions. The equilibrium is then shifted toward the species with the greatest affinity for the target. In an alternative embodiment, the target is added after reversibility has been stopped; this has the advantage that one may use reversible

reactions which are not compatible with the presence of the target but the process is not adaptive and no amplification of the preferred component can result; it is however sufficient for lead generation, i.e. the discovery of components presenting the activity sought for; in its second phase it amounts to the usual, static combinatorial chemistry approach where an actual, real library is screened by the target; the resulting library may be termed pre-equilibrated or postdynamic combinatorial library (pDCL).

In both embodiments a) and b) described above, the target can be used in a soluble form. The target can also be present in immobilized form, for example bound to a solid support. After the generation of the library and the addition of the target (which is present either during the generation of the library or added afterwards, as laid out above) the reaction mixture containing the library is submitted to a work-up procedure which can be carried out in the following ways:

A) The target with the components bound to it is separated from the reaction mixture by methods known to the person skilled in the art. In the case of immobilized targets, the target is simply taken out of the mixture of or filtered off. In the case of a soluble target, the target-component adduct can e.g. be removed by filtration techniques, in particular by ultrafiltration. After the separation of the target-component adduct the components are released. This can occur by destroying the target, for example by denaturation, in the case of a protein. It is sometimes also possible to release the component by changing the pH of the mixture. In particular, in the case where the target is a protein and protein-protein interactions are inhibited, a pH-change, for example by re-dissolving the target in a buffer solution, will release the bound components. After the release, the components are identified.

B) The target remains in the reaction mixture. It may not be necessary to release the bound components from the target, for example in cases when the target can promote formation of preferred species with a turnover. A more specific example for this are reactions in which the compounds of the generated library are transient species which have to be converted into stable species. One precise example are

imines which have to be reduced to amines before analysis. The release of the components is however necessary in many cases. This is generally done with the methods known to the person skilled in the art, in particular with the methods which were described under A). In cases where the components are released, such release can occur simultaneously with the conversion of the transient species into stable species.

In order to identify the compounds which preferably bind to the target and which are hence preferably formed in the target, several possibilities exist. In one embodiment, the library formed in the presence of the target is analysed (i.e. the compounds formed are identified by analytical methods known to the person skilled in the art, preferably by mass spectrometry) and compared with a library formed under identical conditions, but in the absence of the target. The compounds which preferably bind to the target are those which are formed in higher amounts in the presence of the target. This embodiment is preferred when the target is used in soluble form. The target-ligand complex is generally destroyed when the mixture is analysed.

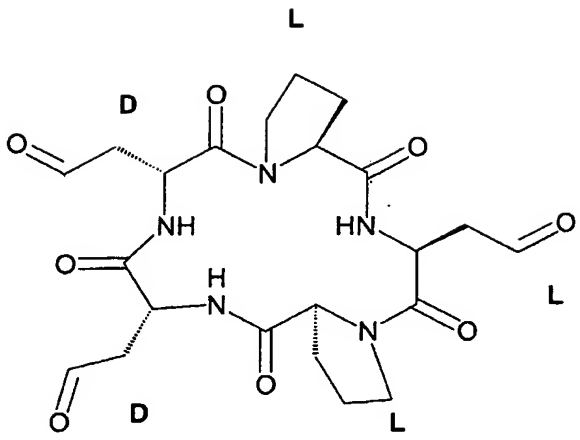
It is also possible to isolate the target-ligand complex from the mixture, release the active compound (ligand) from the target and analyse the ligand. This is preferred in cases the target is used in immobilised form.

In another preferred embodiment of the present invention, the library in generated under conditions under which the constituents of the library (i.e. the building blocks and the compounds generated) are present only in trace amounts which are too low to permit the analysis of the mixture. The presence of the target shifts the equilibrium towards the preferred components; however, to induce an amplification of the formation of the preferred components, a simultaneous reaction is carried out in the reaction mixture which irreversibly converts the components formed into species which can no longer form reversible bonds with the other molecules present. This irreversible conversion reaction has to occur sufficiently slow, in order not to produce significant amounts of the converted species within a certain time laps in the absence of the target. Said time laps depend upon a

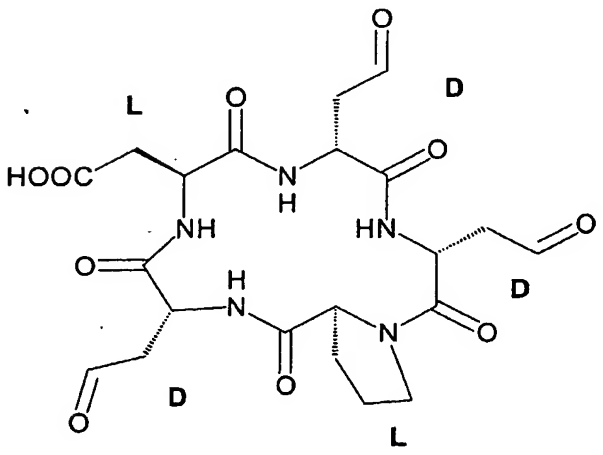
number of parameters like, for example the reaction chosen and its speed of reaction, the concentration of the reaction partners, or the temperature.

Said time laps must in any case be such that the converted species, which are derived from the components which preferably bind to the target, are selectively enriched and can be analyzed. One example for an irreversible conversion reaction is the reaction of amines (formed in a reaction between aldehydes and amines which, by a cleavage of the bond, give the starting linking groups) to the corresponding amines by reducing agents known in the art such as, for example, boron hydrides.

Non-limiting examples of the cyclic oligopeptides according to the invention comprise the molecules **1** to **3** below.



1



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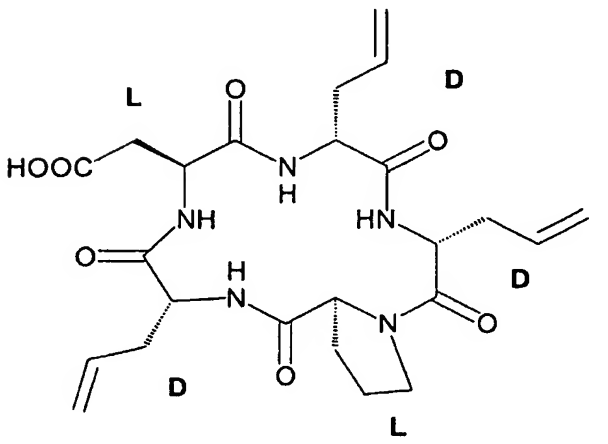


3

Each of these cyclic oligopeptides, which are preferred embodiments of the invention contains a number of linking groups that will dynamically interact with the linking groups in the molecules forming the constituents of the library.of substituents. The linking groups can be of any type compatible with DCL, such as aldehyde/imine, terminal alkene/alkene, thiol/disulfide, borate/borate esters and others. For example, compounds **1** and **2** contain aldehyde side chains that can dynamically form imines upon interactions with mixtures of amines. They can also interact with a mixture of amines of general formula RNH_2 or with acyl hydrazides of general formula $RC(O)NHNH_2$. wherein R is any appropriate organic substituent. Compound **3** can interact with a mixture of alkenes under reversible olefin metathesis conditions to dynamically modify the scaffold with substituents via C=C bonds. The two residues in the D-form in compounds **1** to **3** improve proteolytic stability of the cyclic oligopeptides and also of the active compounds obtained after the screening process. The cyclic peptides **1** to **3** are preferably used as scaffolds to find components which are active in impairing protein-protein-interactions, in particular in the inhibition of protein-protein-interactions.

The present invention will now be illustrated in the following non-limiting examples.

EXAMPLES

Example 1

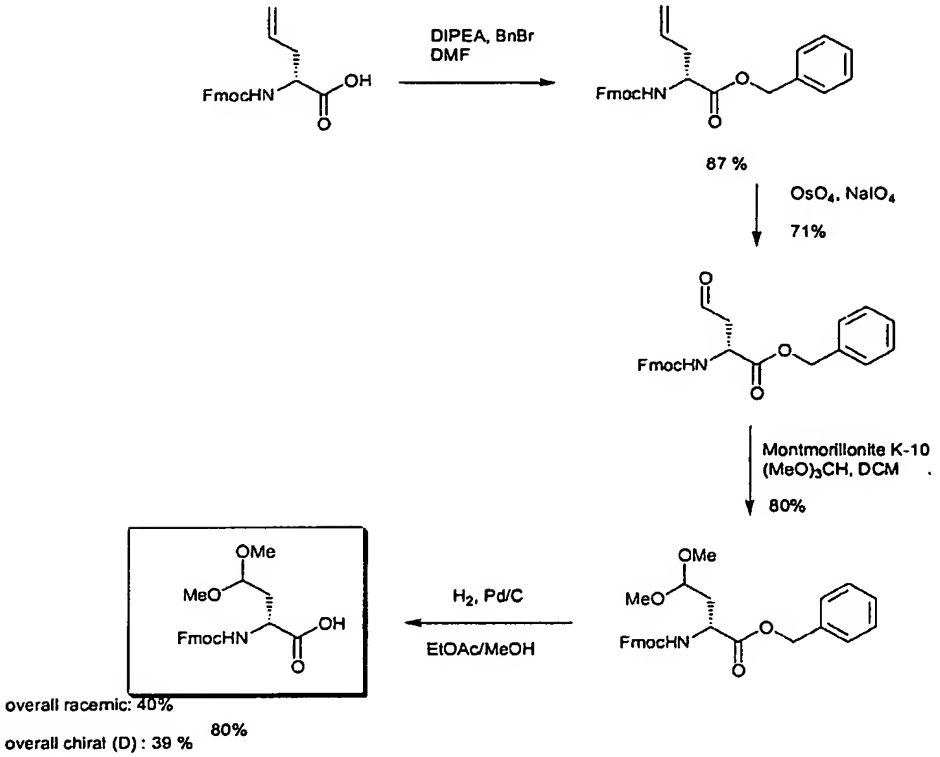
Example 1

Synthesis of the cyclic scaffold **2**

The synthesis of the compound **2** is laid out in the following scheme 1

A: Synthesis of (D)-2-amino-4-dimethoxy butyric acid

Synthesis of (D)-2-amino-4-dimethoxy butyric acid

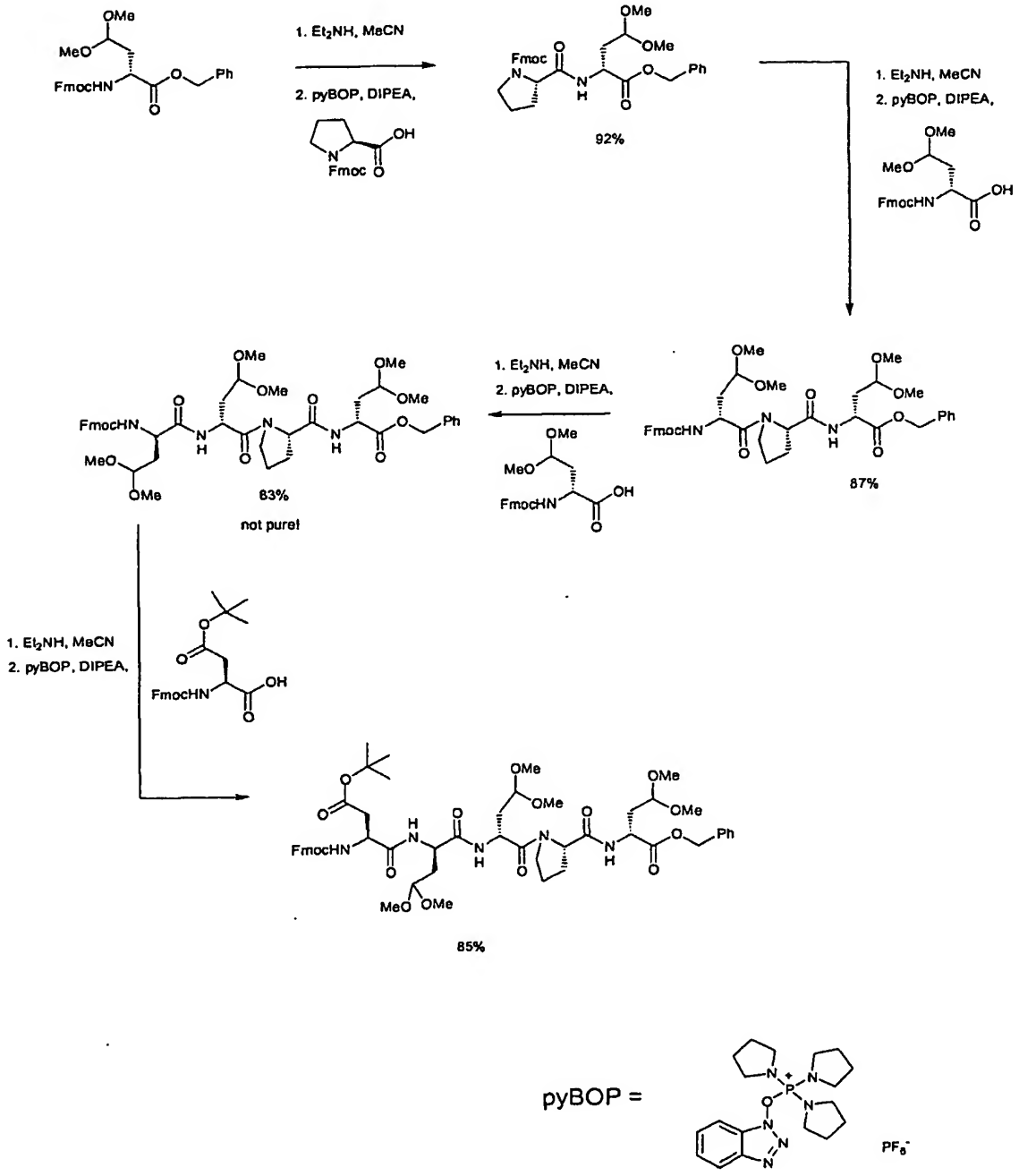


Scheme 1: Synthesis of cyclic scaffold **2** (part 1)

The compounds **1** and **3** are synthesized in an analogous way, with the use of the compounds carrying a carbonyl group or, respectively, an olefin group in place of the aldehyde group. The analytical data were in accordance with the structure.

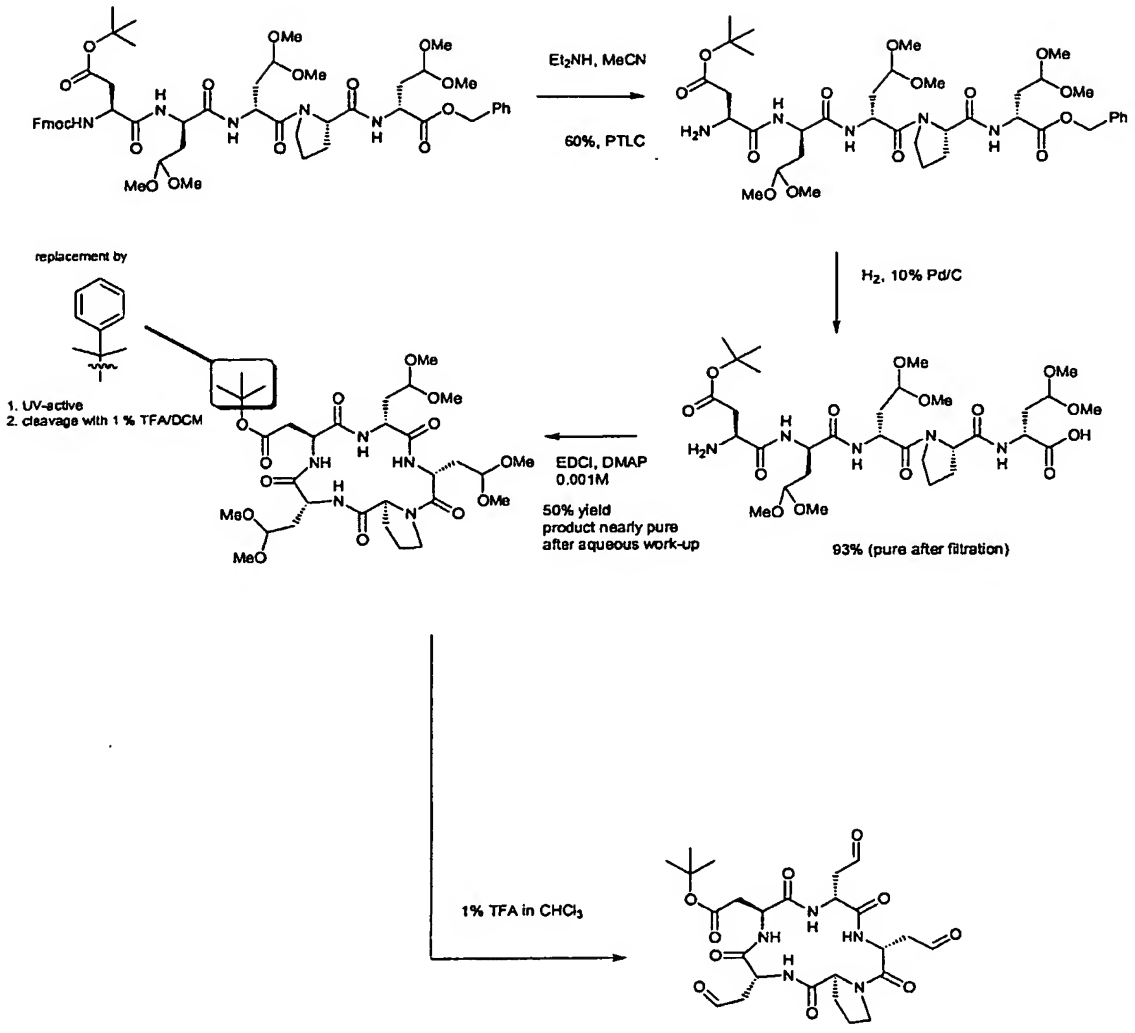
B Synthesis of linear Pentapeptide

Synthesis of linear Pentapeptide



Scheme 3: Synthesis of the cyclic scaffold 2 (part 2)

Cyclisation



Scheme 3: Synthesis of the cyclic scaffold **2** (part 3)

Claims

1. A method of forming a library of components which are potentially capable of binding to a target, which method comprises

   i) selecting a plurality of molecules carrying a functionality which may interact with a binding site of the said target, said molecules furthermore having a linking group which is capable of interacting with other linking groups under the formation of reversible bonds;

   ii) selecting a scaffold carrying two or more linking groups which are capable of interacting with the linking groups on the compound carrying a functionality;

   iii) reacting the scaffold and the molecules carrying the functionality in the presence of the target, under conditions where a formation of reversible bonds between the functionalities on the scaffold and on the molecules carrying a functionality occurs.

2. The method according to claim 1, wherein, before the target is added in step iii), a step ii)a) is conducted in which the scaffold and the molecules carrying the functionality are reacted in the absence of the target until equilibrium is reached.

3. The method according to claim 1 or 2, wherein the scaffold is biologically inactive.

4. The method according to claim 1 or 2, wherein the scaffold is biologically active.

5. The method according to any of the claims 1 to 4, wherein the reversible bonds formed between the linking groups present on the scaffold and on the molecules carrying the functionality are covalent bonds.

6. The method according to claim 5, wherein the linking groups present on the scaffold and on the molecules carrying the functionality are selected from the group consisting of amino groups, aldehyde groups, keto groups, thiol groups, olefinic groups, alcohol groups, carbonyl groups, hydrazine groups, hydroxylamine groups and borate groups.

7. The method according to claim 6, wherein the linking groups react under the formation of imines, oximes, acylhydrazones, amides, acetals, esters, borate esters, disulphides or olefinic bonds.

8. The method according to any of the claims 1 to 4, wherein the reversible bonds formed between the linking groups present on the scaffold and on the molecules carrying the functionality are supramolecular bonds.

9. The method according to any of the claims 1 to 8, wherein the molecules forming the library are present in amounts which are too low to permit an analysis of the mixture and wherein, after the addition of the target, the components formed are irreversibly converted into species which can no longer form a reversible bond with the molecules present in the reaction mixture.

10. The method according to claim 9, wherein the irreversible conversion reaction is so slow as not to produce significant amounts of the converted species within a certain time laps, in order to ensure an enrichment in those converted species which are derived from the components which preferably bind to the target, and after which enrichment the converted species can be analyzed.

11. The method according to any of the claims 1 to 10, wherein the structure of the target and/or its binding site is known and the scaffold is designed to interact with the target.

12. The method according to claim 11, wherein the scaffold is a non-cyclic or cyclic hydrocarbon molecule which can contain one or more heteroatoms, preferably from the group consisting of N, O, S, P, B, Si and halogens, and which can contain one or more double or triple bonds.

13. The method according to claim 12, wherein the scaffold contains at least two linking groups.

14. The method according to claim 13, wherein the scaffold contains two to six linking groups

15. The method according to claim 14, wherein the scaffold contains two to four linking groups.

16. The method according to claim 15, wherein the scaffold contains three or four linking groups.

17. The method according to claim 12, wherein scaffold is an oligopeptide or an oligopeptide derivative having a peptide-like three-dimensional structure.

18. The method according to claim 17, wherein the scaffold is an aromatic or non-aromatic cyclic hydrocarbon molecule or it is based on an aromatic or non-aromatic cyclic hydrocarbon molecule.

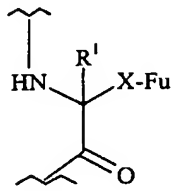
19. The method according to claim 18, wherein the scaffold is selected from mononuclear cyclic compounds and fused bi- to quadronuclear cyclic compounds which can contain one or more heteroatoms from the group consisting of N, O, and S.

20. The method according to claim 19, wherein the scaffold is selected from 4 to 7-membered aromatic or non-aromatic rings which can contain in their cycle up to three hetero atoms from the group consisting of O, S and N.

21. The method according to claim 20, wherein the scaffold is selected from aromatic and non-aromatic 6-membered carbocyclic rings.

22. The method according to any of the claims 1 to 10, wherein the structure of the target and/or its binding site is not known.

23. The method according to claim 22, wherein the scaffold is a cyclic oligopeptide according to the general formula



I

in which

Fu is a linking group selected from -C(O)H, -C(O)R^2, -C(O)OM, -CR^3=CH_2, -C≡CH, NHR^4, - $N(R^6)$-NHR^5, - SH, -OH and -$B(OH)_2$, and which linking groups Fu are identical or different from each other in case two or more of them are present in the cyclic oligopeptide;

X is a single or a double bond or a C_1-C_5-alkylene group in which one or more methylene groups can be replaced by O, S or NR^7;

R^1 is H or a C_1-C_4-alkyl group; or R^1 forms together with the N-atom in beta-position a 5- to 6- membered heterocycle under replacement of the H-atom attached to the said N-atom:

R^2 is a C_1-C_6-alkyl group;

R^3 is selected from the group consisting of H; C_1-C_{10}-alkyl; phenyl, -CH_2(phenyl), -CH_2CH_2(phenyl), phenoxy, heteroaryl -CH_2(heteroaryl), -CH_2CH_2(heteroaryl) and heteroaroxy;

R^4, R^5 and R^6 are independently of each other selected from the group consisting of H; unsubstituted and at least monosubstituted C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl and C_2-C_{10}-alkynyl, the substituents of which are selected from the group consisting of halides, OH, C_1-C_6-alkoxy, (C_1-C_6-alkyl)mercapto, CN, $COOR^8$, $CONR^9R^{10}$, unsubstituted and at least monosubstituted phenyl and heteroaryl, the substituents of which are selected from the group consisting of halides, pseudohalides, C_1-C_3-

alkyl, C_1-C_3-alkoxy and CF_3; and unsubstituted and at least monosubstituted phenyl and heteroaryl, the substituents of which are selected from the group consisting of halides, pseudohalides, C_1-C_3-alkyl, C_1-C_3-alkoxy and CF_3;

R^7 is H or a C_1-C_6-alkyl group;

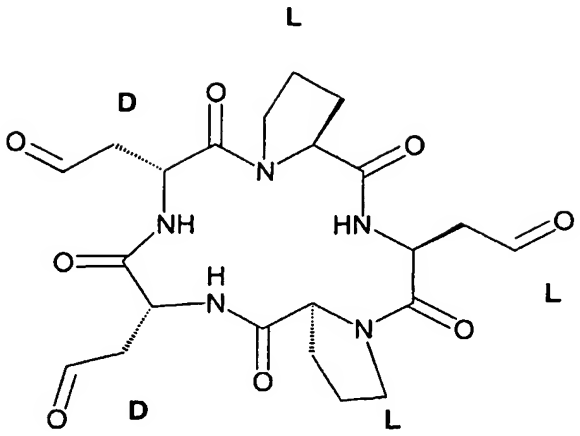
R^8 is H, C_1-C_6-alkyl or benzyl;

R^9 is selected from the group consisting of: H; C_1-C_6-alkyl which can be phenyl-substituted; and phenyl; and wherein each of the aforementioned aromatic groups can be unsubstituted or carry one or more substituents from the group consisting of halides, pseudohalides, C_1-C_3-alkyl, C_1-C_3-alkoxy and CF_3;

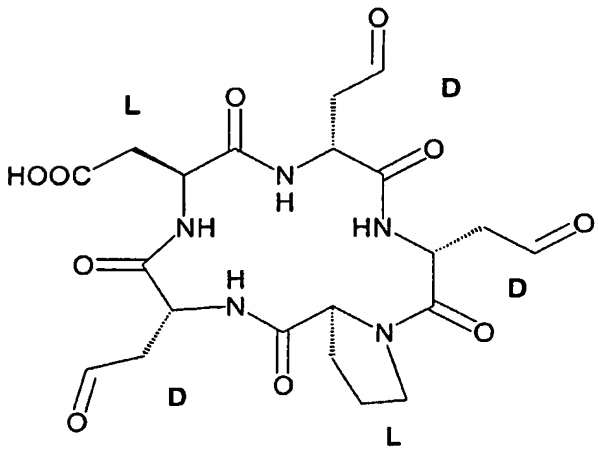
R^{10} independently has the same meaning as R^9;

M is selected from the group consisting of H; alkali metals; alkaline earth metals and ammonium groups which may carry one or more organic substituents.

24. A method as defied in claim 23 wherein one of the cyclic peptides 1 to 3



1



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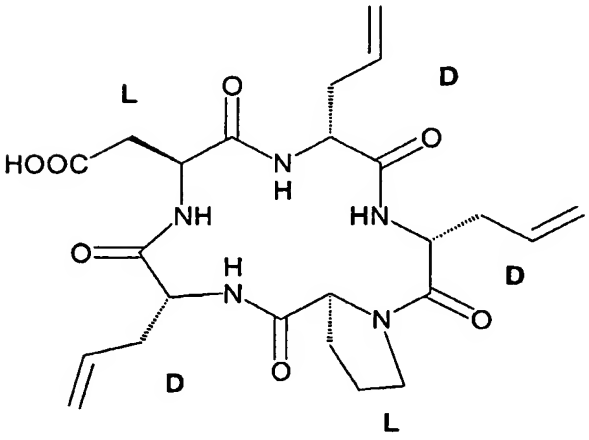


3

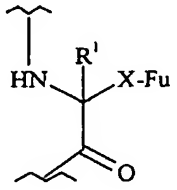
is used.

25. The method according to claim 24, wherein the cyclic peptides **1** to **3** are to find components which are active in impairing protein-protein-interactions, in particular in the inhibition of protein-protein-interactions.

26. A method of assessing the binding capacity of a component to bind to a given target, which method comprises carrying out the method as described in any of the claims 1 to 25, and which method additionally comprises

iv) identifying the components which preferably bind to the target

27. A cyclic oligopeptide of the formula



I

in which

Fu is a linking group selected from -C(O)H, -C(O)R^2, -C(O)OM, -CR^3=CH_2, -C≡CH, NHR^4, - N(R^6)-NHR^5, - SH, -OH and -$B(OH)_2$, and which linking groups Fu are identical or different from each other in case two or more of them are present in the cyclic oligopeptide;

X is a single or a double bond or a C_1-C_5-alkylene group in which one or more methylene groups can be replaced by O, S or NR^7;

R^1 is H or a C_1-C_4-alkyl group; or R^1 forms together with the N-atom in beta-position a 5- to 6- membered heterocycle under replacement of the H-atom attached to the said N-atom:

R^2 is a C_1-C_6-alkyl group;

R^3 is selected from the group consisting of H; C_1-C_{10}-alkyl; phenyl, -CH_2(phenyl), -CH_2CH_2(phenyl), phenoxy, heteroaryl -CH_2(heteroaryl), -CH_2CH_2(heteroaryl) and heteroaroxy;

R^4, R^5 and R^6 are independently of each other selected from the group consisting of H; unsubstituted and at least monosubstituted C_1-C_{10}-alkyl, C_2-C_{10}-alkenyl and C_2-C_{10}-alkynyl, the substituents of which are selected from the group consisting of halides, OH, C_1-C_6-alkoxy, (C_1-C_6-alkyl)mercapto, CN, $COOR^8$, $CONR^9R^{10}$, unsubstituted and at least monosubstituted phenyl and heteroaryl, the substituents of which are selected from the group consisting of halides, pseudohalides, C_1-C_3-alkyl, C_1-C_3-alkoxy and CF_3; and unsubstituted and at least monosubstituted phenyl and heteroaryl, the substituents of which are selected from the group consisting of halides, pseudohalides, C_1-C_3-alkyl, C_1-C_3-alkoxy and CF_3;

R^7 is H or a C_1-C_6-alkyl group;

R^8 is H, C_1-C_6-alkyl or benzyl;

R^9 is selected from the group consisting of : H; C_1-C_6-alkyl which can be phenyl-substituted; and phenyl; and wherein each of the aforementioned aromatic groups can be unsubstituted or carry one or more substituents from the group consisting of halides, pseudohalides, C_1-C_3-alkyl, C_1-C_3-alkoxy and CF_3;

R^{10} independently has the same meaning as R^9;

M is selected from the group consisting of H; alkali metals; alkaline earth metals and ammonium groups which may carry one or more organic substituents.

28. The cyclic peptides 1 to 3 as defined in claim 24.